SOME PHYSIOLOGICAL PROPERTIES OF DEXTRAL AND OF SINISTRAL FORMS IN BACILLUS MYCOIDES FLÜGGE

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The attention of biologists has long been concentrated on the study of dextral and of sinistral forms in populations of the same species. Recently Ludwig (1932, 1936) in two extensive reviews has summarized the numerous scattered observations in this field. Among studies of dextrality and sinistrality in the broad sense it seems useful to treat separately a distinct group of observations on spiral structures which are spread widely in different representatives of both plant and animal kingdoms. One of the basic attributes of spiral structures is their property of undergoing genotypic inversion with comparative ease, as has been shown, for instance, by Boycott, Diver, Hardy and Turner (1929) in the mollusk *Limnaea peregra.* In almost all cases when experimental work is carried out with organisms possessing a spiral form, it is possible to detect among the individuals of typical structure a few hereditary inverse specimens.

It may be supposed that the direction of the spiral is determined by some substance which is labile in the sense that it is able to undergo inversion of configuration with comparative ease. Such an idea is due to Koltzoff (1934) and to Needham (1934), who wrote that it is possible that the origin of dextrality and sinistrality in the eggs of certain snails, which later appear in the direction of spiral twist of their coil, is due to sterochemic properties of the protein molecules composing them. At present these relations are very obscure, and it seems essential to record accurately which physiological properties of dextral and sinistral spiral forms of organisms are identical and which are not. Such analysis is a necessary step in the attempt to comprehend the mechanism of morphologic inversions.

The Material and its Morphologic Properties

For the investigation of physiological peculiarities of dextral and sinistral spiral forms *Bacillus mycoides,* described by Flügge in 1886, was chosen. Various cultures of dextral and sinistral forms of this bacterium have been received from the Microbiologic Institute of the

Academy of Sciences (Moscow). Under natural conditions a form of *Bacillus mycoides* is usually met with which, when grown on the surface of agar-peptone medium, produces colonies spirally twisting to the left, i.e., counter-clockwise. The inverse form of this microbe, producing dextral coils in growing, rarely occurs. Isolated colonies consisting wholly of dextrally twisted individuals are sometimes found in soils. The dextral form of *Bacillus mycoides* was first recorded by Gersbach (1922), and in experiments with agglutination he established that the dextral and sinistral strains of this organism are perfectly identical in all their properties. Later dextral strains among a number of sinistral ones were observed by Oesterle (1929) and by Lewis (1932). The dextrality and sinistrality in *Bacillus mycoides* is a hereditary feature inasmuch as dextral forms are always obtained from dextral forms and sinistral forms from sinistral after numerous transfers on peptone-agar.

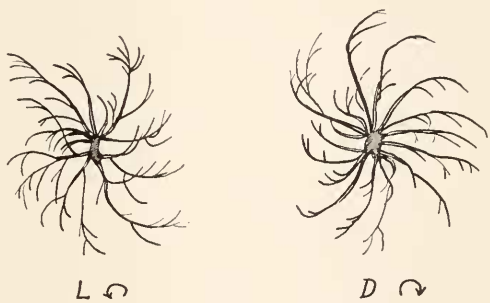

FIG. 1. Dextral (*D*) and sinistral (*L*) colonies of *Bacillus mycoides,* 18 hours after inoculation on peptone-agar in Petri dishes, at 29° C. Diameter of the colonies attains 20 mm.

Figure 1 represents the appearance of colonies formed by the dextral and sinistral forms of *Bacillus mycoides* on peptone-agar. With the introduction of a small quantity of inoculating material into the centre of a Petri dish of agar-peptone, the growth begins and the thin filaments of the growing culture of the L-form steadily deviate to the left and those of the D-form to the right.

It can be shown that the spiral form of the colony of *Bacillus mycoides* is a secondary feature which is the result of the primary spiral structure of the growing cells of the filament. If one stains the growing cells of the filaments on the surface of agar by neutral red or by toluedin blue (1 : 5000), and then examines them under the microscope, one sometimes detects the twisting of two filaments on encountering one another on the surface of the agar. A number of such observations prove that during the growth of the filament motions of two kinds take place simultaneously: first, the elongation and, second, the twist around the axis of the filament. As a result of the combination of these two motions, the structure of the individual cell and with it the structure of the whole filament forms a spiral. Similar observations have been made also by Stapp and Zycha (1931) and by Roberts (1938).

If during the free growth of a filament on the agar surface, the filament rotates around its longitudinal axis counter-clockwise, as a result of interaction with the firm surface of the agar the growing filament will also steadily coil in a counter-clockwise direction. Consequently, the secondary sinistral coil of the growing colony of bacteria will arise as a result of the primary sinistral spiral growth of the cells of the filament. This viewpoint is confirmed by the following observations. It appears that a certain consistency of culture medium is necessary for the typical spiral growth of colonies of *Bacillus mycoides* (Pringsheim and Langer, 1924; Hastings and Sagen, 1933). The latter authors state that on agar of usual strength the growth of *Bacillus mycoides* spreads from the place of inoculation in the form of coarse filaments which twist counter-clockwise, forming a symmetrical pattern. On less consistent agar this pattern disappears, and the growth becomes diffuse.

Methods

The strains used in this investigation were cultivated during the last five years under standard laboratory conditions. It is recorded that long cultivation and repeated transfer of the strains of *Bacillus mycoides* level many differences in morphological and physiological characteristics observed immediately after the isolation of the strains from natural conditions (Kononenko, 1935), and only those of them are retained which are due to the hereditary pattern of organization of the strain. This circumstance is of significance in the comparison of physiological properties of dextral and sinistral forms.

Experiments were made under sterile conditions. The bacteria were cultivated in Petri dishes on a solid medium of the following composition: K_2HPO_4—0.05 per cent; $MgSO^4$—0.03 per cent; NaCl—0.03 per cent, peptone—1 per cent and agar-agar—2 per cent. In some experiments the effect of optical isomers of amino acids upon the bacteria was studied.[1]

[1] In this work preparations of the following origins were used: *d*-valine, *l*-valine, *l*-aspartic acid and *dl*-aspartic acid: Schuchardt Co. (Görlitz); *d*-leucine (reinst), *l*-leucine (reinst), *l*-histidine monochlorhydrate, *d*-histidine monochlorhydrate, *l*-β-phenyl-α-alanine (reinst), *d*-β-phenyl-α-alanine (reinst): Fraenkel and Landau (Berlin); *d*-arginine monochlorhydrate (recrystallized from 80° alcohol), *dl*-arginine monochlorhydrate (isolated by Dr. M. Lissitzin): Protein Laboratory of the Academy of Sciences (Moscow).

Relations of Growth in Dextral and in Sinistral Strains to Temperature

The first group of experiments was devoted to the study of the effect of temperature on the growth of dextral and sinistral strains of *Bacillus mycoides*. Before the inoculation *Bacillus mycoides* was always cultivated on solid agar in Petri dishes at 20° C., while for the last forty-eight hours the culture was maintained at 15° C. From such cultures, accustomed to moderate temperatures, an inoculation into solid or liquid medium was made and the growth was followed usually at the following temperatures: 20°, 24°, 28° and 32° C. In cases when it is not specially mentioned, the experiments were made with the sinistral strain, L–1, and with the dextral strain, D–1. The basic conclusions arrived at in the work with these strains were further confirmed upon other strains, as will be described below.

Figure 2 gives the results of three series of experiments made in Petri dishes. Into the centre of each dish was introduced a small quantity of inoculating material which formed a circle with a diameter of 0.5 mm. Twenty hours after the beginning of the growth the diameter of the colony was measured and as a rule it had attained about 6 mm. at 20° C., and about 25 mm. at 32° C.

Observations have shown that at this time the growth proceeds at all temperatures according to a geometric progression. In Fig. 2 the size of colonies of the dextral and sinistral strains growing at 20° C. was taken as a unit, and the size at other temperatures is expressed in relative values. The fundamental conclusion which may be drawn from Fig. 2 is that the sinistral and dextral strains of *Bacillus mycoides* differ sharply from one another in the relation of growth to temperature. If the rate of growth of the usual sinistral strain increases exponentially with the rise of temperature, as is typical for the majority of biological processes, in the inverse dextral strain the phenomenon of " heat injury " may be observed. With the rise of temperature the velocity of growth of the dextral strain increases always more and more slowly.

This observation has necessitated a more thorough study of the phenomenon of " heat injury " in the dextral strain in additional material. In the preceding experiments the growth of the so-called R-forms of bacteria, producing sinistral (LR) or dextral coils (DR) was studied. As a result of " dissociation " both dextral and sinistral strains of *Bacillus mycoides* develop into smooth ones, the so-called S-forms, deprived of coiling and forming during their growth on solid medium flat spheric colonies (LS and DS) (for literature see Arkwright, 1930). In their external appearance S-forms of dextral and sinistral strains are certainly indistinguishable from one another. Investigations devoted to the study of dissociation in *Bacillus mycoides* have shown that the transition of the R-form into S-form is very easily accomplished, while the reverse transformation of the S-form into the R-form is extremely

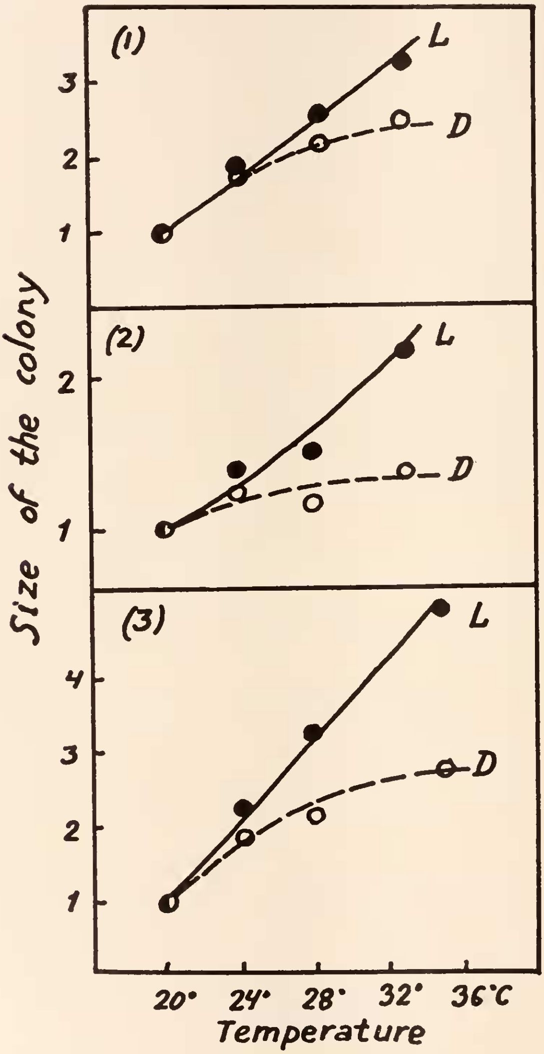

FIG. 2. Temperature relation of growth in the dextral (*DR*) and sinistral (*LR*) strains of *B. mycoides* on the solid medium. Each point represents an average for three dishes.

unusual (Stapp and Zycha, 1931; Lewis, 1932; Dooren de Jong, 1933).

An attempt was made to find out whether the specific differences in the dependence of growth on temperature in the dextral and sinistral strains which were observed on the R-forms will also be maintained in the case of the S-forms. Experiments on the relation of growth to temperature in LS and DS forms of *Bacillus mycoides* growing on solid

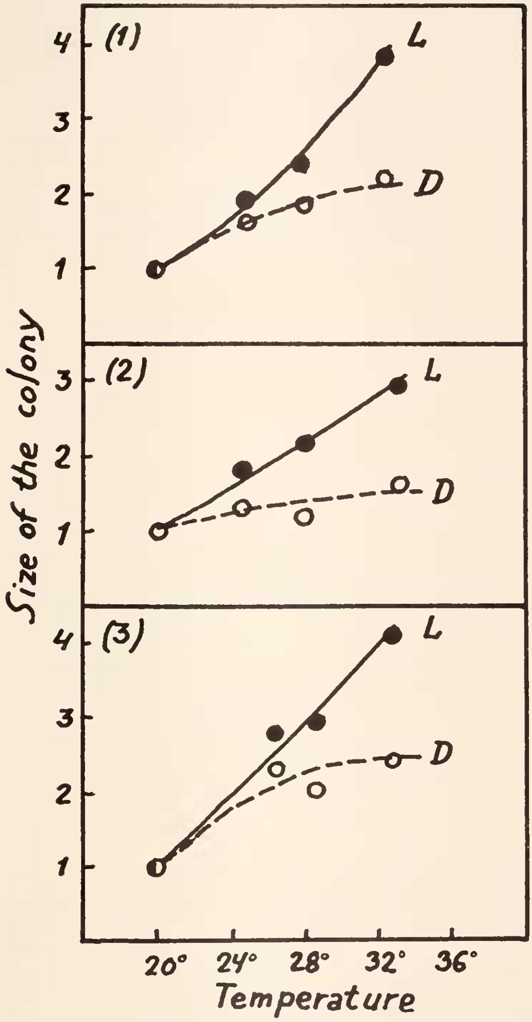

FIG. 3. Temperature relation of growth in the smooth dextral (*DS*) and sinistral (*LS*) strains of *B. mycoides* on the solid medium. Each point represents an average for three dishes.

medium were made according to the previous plan and the results of the three series of observations are given in Fig. 3. It is evident that the phenomenon of heat injury in growth which was found to be characteristic for the dextral strains is practically equally marked both in the R-form and in the S-form of the bacteria.

Further experiments on the relation of growth to temperature in DS and LS strains were made with liquid medium of the following composition: K_2HPO_4—0.05 per cent, $MgSO_4$—0.03 per cent, NaCl—0.03 per cent, glucose—1.0 per cent, d-arginine—0.05 per cent. The medium in this and in further experiments was sterilized without arginine, and a powder of arginine was aseptically added to it later. The bacteria were cultivated in test-tubes previously sterilized by dry heat and containing 1 cc. of aseptically added sterile culture medium. The inoculating material was prepared in the following manner. Two loops of

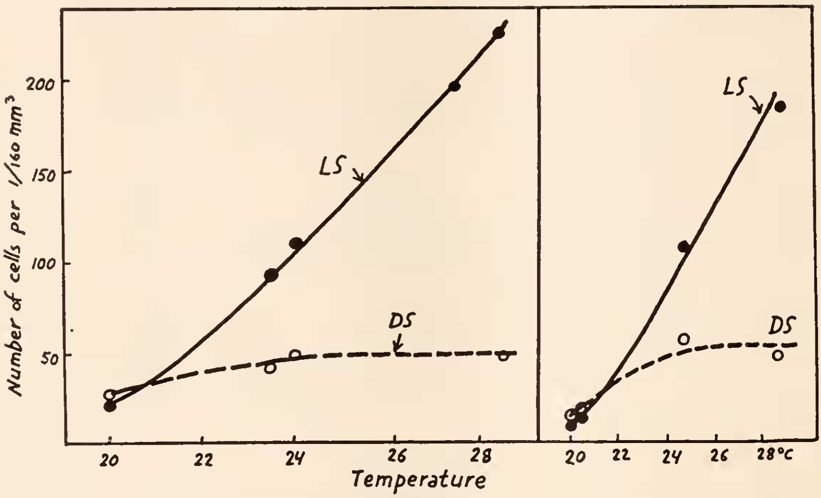

FIG. 4. Temperature relation of growth in the *DS* and *LS* strains of *Bacillus mycoides* in the liquid medium. Each point represents an average for four observations.

bacteria growing in Petri dishes on agar-peptone at 20° C. were shaken up in 3 cc. of sterilized bidistilled water, and two drops of this mixture were aseptically introduced into each of the experimental test-tubes, which were subsequently kept at different temperatures (20°, 24°, 26° and 28° C.). The measurement of growth consisted in counting the number of cells in a Thoma chamber under the microscope forty hours after the inoculation. In Fig. 4 the relation of growth to temperature is given as observed in two series of experiments upon the LS and DS forms of *Bacillus mycoides* in the liquid medium. It may be pointed out that in the liquid medium the velocity of growth of the sinistral strain rises exponentially with the increase of temperature, while in the dextral strain the characteristic heat injury is distinct in the range 24°–28° C

In the light of all these experiments it is certain that the dextral (D–1) and sinistral (L–1) strains of *Bacillus mycoides* under examination possess distinctly different relations of growth to temperature and that these distinctions are retained both in solid and in liquid medium, both in R and S modifications. However, are these observations sufficient for the belief that the differences observed are actually connected with the dextrality and sinistrality of the strains studied? In order to

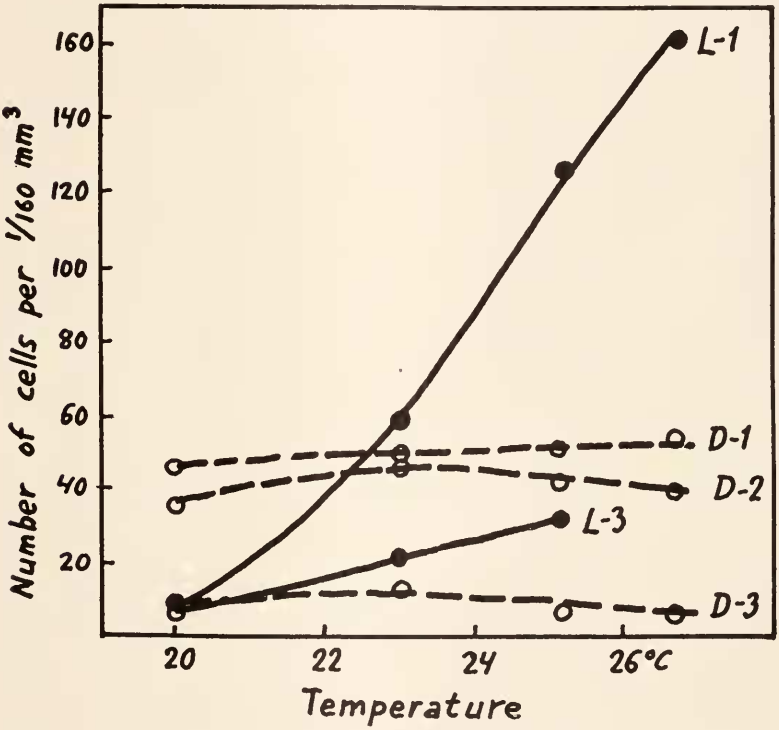

FIG. 5. Temperature relation of growth in the smooth sinistral (*L–1, L–3*) and dextral (*D–1, D–2, D–3*) strains of *Bacillus mycoides* in the liquid medium. Each point represents an average for three observations.

answer this question it is necessary to observe a great number of various strains of *Bacillus mycoides*. To this end one additional sinistral strain, L–3, and two new dextral inverse strains, D–2 and D–3, were chosen. All these strains had been cultivated under laboratory conditions for five years. S-modifications of various strains were used for experimentation for the reason that it is easier to prepare the suspension for inoculation from them. The results of experiments carried out in liquid medium with five strains of *Bacillus mycoides* of different origin are given in Fig. 5. It may be pointed out that the differences in the relation of growth to temperature are more distinctly expressed in the two strains which were previously compared, i.e., in L–1 and D–1. However, if one examines the temperature range 23°–26° C., it may be observed that there is a specific distinction between all the dextral and all the sinistral strains. In dextral strains (D–1, D–2, D–3) in this temperature range the rate of growth does not rise with the rise in temperature, while in both sinistral strains (L–1 and L–3) the rate of growth rises considerably with the increase in temperature.

Physiological Difference between the Sinistral Strains and the Dextral Strains of Bacillus mycoides (Lewis Reaction)

Lewis (1933), working in Texas with dextral and sinistral strains of *Bacillus mycoides,* recorded a specific physiological distinction between them. It is known that *Bacillus mycoides* possesses the capacity of decomposing glucose and saccharose with the formation of acids. The production of acids on glucose is similar in sinistral and in dextral strains. This similarity does not hold true for the decomposition of saccharose. Lewis writes that the rapid formation of acid on saccharose is, on the whole, connected with the sinistral direction of spiral growth, while the arrest of this reaction is correlated with the dextral twist of the filaments.

It was decided to repeat these experiments of Lewis with the strains of *Bacillus mycoides* under investigation. S-forms of different strains were used in these experiments. Two per cent of saccharose was added to agar-peptone of usual composition and also some weak (0.02 per cent) solution of phenol red (according to Clark). The latter had an orange tint at pH 7.7, which is optimal for the growth of *Bacillus mycoides.* The microbes were cultivated at 28° C. Nineteen hours after the beginning of the experiment the color of the sinistral strains differed very sharply from that of the dextral strains. The sinistral strains, L–1 and L–3, showed rapid production of acid on sucrose. The reaction of the colony was distinctly acid to phenol red (pH about 6.8). The dextral strains, D–1, D–2 and D–3, showed no production of acid on sucrose. The reaction of the colony was alkaline to phenol red (pH about 8.4).

The results of Lewis are therefore confirmed and it may be pointed out that we used S-modifications while he used R-modifications of the bacteria. This confirmation includes also the following detail. The dextral strains are, without exception, unable to produce acid on sucrose, while almost all sinistral strains readily form acid on sucrose. To this general rule, Lewis noted one exception and I have also come across one doubtful case.

The reaction of Lewis may be considered to be a certain fermentative weakness of inverse dextral strains as compared to typical sinistral ones.

On the Relation of Morphological Inversion to Molecular Inversion

The phenomenon of heat injury of inverse dextral strains of *Bacillus mycoides* coincides in a remarkable way with the heat injury taking place on cultivating different lower organisms on unnatural isomers of amino acids described by Gause and Smaragdova (1938). When the yeast cells *Torula utilis* are grown on the usual isomer of leucine, which enters into the composition of all living organisms, the velocity of growth rises exponentially with an increase of temperature, but when they are cultivated on the unnatural isomer of leucine the velocity of growth increases always more and more slowly with the rise of temperature.

It was decided to repeat this observation of Gause and Smaragdova (1938) on different amino acids and on different organisms. The experiments were made on the growth of *Torula utilis* on optical isomers of leucine, hystidine, phenyl-alanine and valine. Cultivation took place in sterile test-tubes containing 1 cc. of liquid medium of the following composition: KH_2PO_4—0.06 per cent, $MgSO_4$—0.03 per cent, glucose —1.0 per cent, amino acid—0.4 per cent (the latter being aseptically introduced into the sterile medium). The inoculating material was taken from the solid medium, and from it was made a mixture of standard density (50 cells in 1/160 cu. mm.), a few drops of which was then added to liquid in the test-tubes. The counting of the number of cells in the Thoma chamber was made 40 hours after the beginning of the experiment. According to Gause and Smaragdova (1938) this time lies within the limits of the logarithmic phase of growth of *Torula*.

The results of our experiments are given in Fig. 6. Typical heat injury in the temperature range of 18°–20° may be observed in the growth of *Torula utilis* on unnatural isomers of leucine and of histidine. In the case of growth of unnatural isomers of valine and phenyl-alanine such injury is not observed.

Further experiments were made with the growth of *Aspergillus niger* on optical isomers of leucine and valine. The composition of culture medium was as follows: KH_2PO_4—0.35 per cent, $MgSO_4$—0.17 per cent, glucose—1 per cent, amino acid—0.4 per cent. Cultivation was conducted under sterile aerobic conditions in 2 cc. of the liquid. Seventy hours after the beginning of the experiment the wet weight of the mycelium of *Aspergillus,* which was previously drained on filter paper, was measured. The results of experiments given in Fig. 7 coincide completely with the observations made on *Torula utilis.*

Summarizing the results heretofore described, it is evident that during the growth of different lower organisms on optical isomers of certain amino acids the effect of temperature on the growth on natural and on

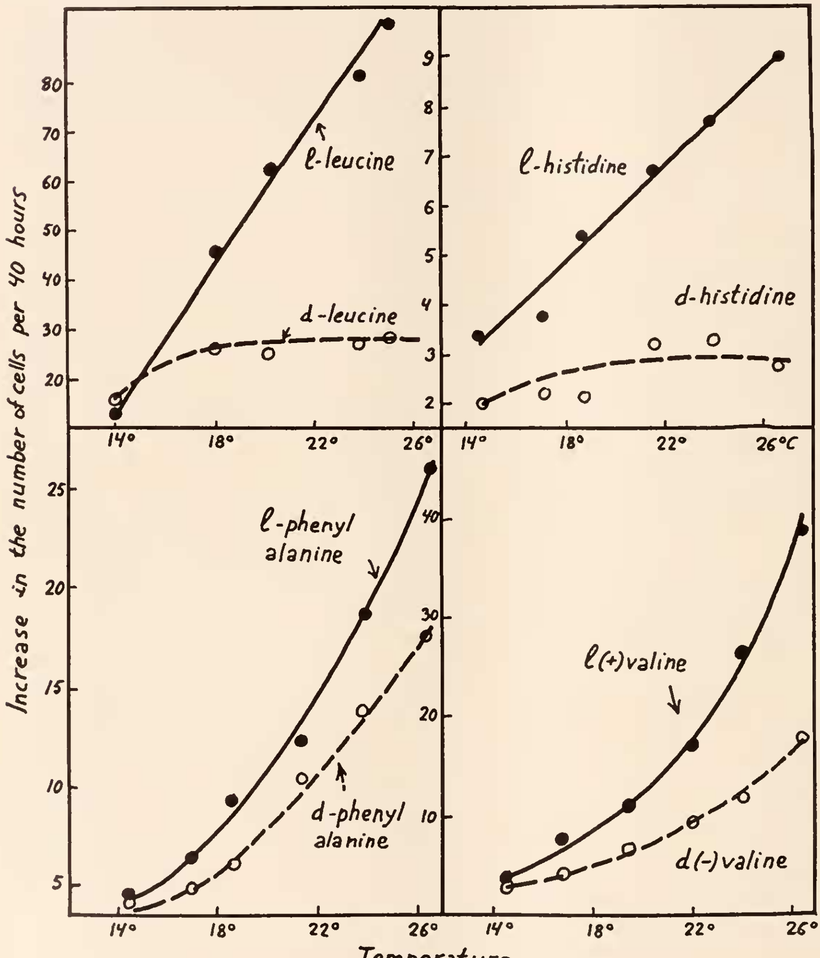

FIG. 6. Growth of the yeast *Torula utilis* on optical isomers of various amino-acids at different temperatures. On the ordinates is plotted the increase in the number of cells per 40 hours in relation to control (the initial density at inoculation, which is taken for a unit). Each point represents an average for two similar cultures.

unnatural isomers proves to be different. The velocity of growth on natural isomers rises exponentially with an increase of temperature. On the other hand, in the same temperature range, the velocity of growth on the unnatural isomer of some amino acids increases with the rise of temperature always more and more slowly. A similar temperature-velocity curve was observed in the inverse dextral strains of *Bacillus mycoides* while they were being grown on the natural substrates. It follows from this type of curve that there is an inhibitive factor in growth and that the intensity of the inhibition rises with the rise of temperature.

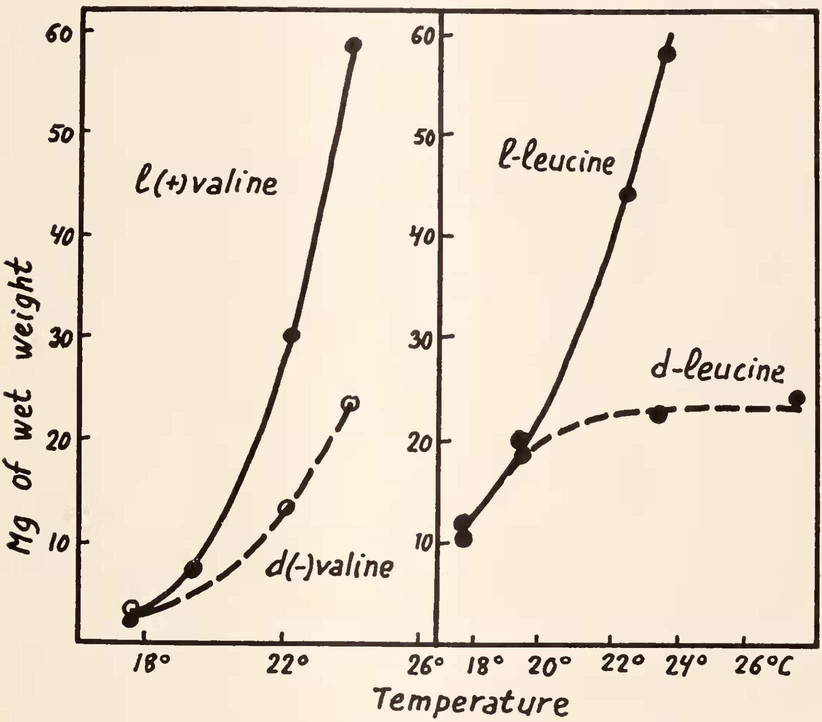

FIG. 7. Growth of *Aspergillus niger* on optical isomers of leucine and valine at different temperatures. Each point represents an average for two similar cultures.

It may be conjectured that in the first series of investigations the unnatural isomer of amino acid dissolved in the culture medium surrounding the cell causes a depression of growth because its steric configuration does not coincide with the steric configuration of the basic constituents of protoplasm. In the second series of investigations one may suppose that the depression of growth in the morphologically inverse form takes place because inside the cell there is an inverse of the normal optical isomer of some organic substance participating in the determination of the form of cells. The depression of growth could thus take place because the steric configuration of the organic substance in question does not coincide with the steric configuration of other constituents of protoplasm.[2]

Growth of Dextral and Sinistral Strains of Bacillus mycoides on Optical Isomers of Certain Amino Acids

All further experiments were carried out with the strains L–1 and D–1. It is to be pointed out that not all amino acids by far are adequate for the nutrition of *Bacillus mycoides.* Thus, for instance, on aspartic

Table I

The growth of sinistral (LR) and of dextral (DR) forms of *Bacillus mycoides on* optically isomeric arginines.

Amino acid	Number of cells in LR	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit	Number of cells in DR	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit
d-arginine......	31.3	1.99	19.7	1.59
dl-arginine.....	15.7		12.4	
d-arginine.....	28.9	1.98	21.2	1.61
dl-arginine.....	14.6		13.2	

acid only a small increase of the number of cells at the beginning of cultivation can be observed, and the further growth soon stops. The experiments have shown that on l-aspartic acid both the smooth sinistral (LS) and the smooth dextral (DS) strains of *Bacillus mycoides* grow better than on the racemic dl-aspartic acid. Among the amino acids used by us the only substance giving unlimited growth of *Bacillus mycoides* was arginine.

The experiments were made at 24° in test tubes with liquid medium of the following composition: K_2HPO_4—0.05 per cent, $MgSO_4$—0.03 per cent, NaCl—0.03 per cent, glucose—1 per cent and arginine—0.05 per cent. Each number given in Tables I and II is a mean value for five parallel observations. In all cases the number of cells in 1/160 cu. mm. was counted 48 hours after the beginning of the experiment.

Both the dextral and sinistral forms of *Bacillus mycoides,* in both twisted and smooth modifications, grow better on natural d-arginine

[2] This hypothesis is further supported by recent observations of Bruckner and Ivanovics (*Zeitschr. f. physiol. Chem.,* **247**: 281, 1937). They observed that in the composition of the capsule of some bacteria an unnatural isomer of glutamic acid participates.

than on the racemic dl-arginine. Since racemic arginine in weak watery solution consists of the dextrorotatory and of the laevorotatory isomers, the lessened growth of *Bacillus mycoides* on this preparation may be explained by the fact that the unnatural laevorotatory isomer, entering into the composition of racemic arginine, is less appropriate for growth than the usual dextrorotatory isomer of arginine. In this relation the properties of dextrally and of sinistrally twisted strains coincide with one another.

Oxidation of Glucose by Dextral and Sinistral Strains of Bacillus mycoides at Different Temperatures

Experiments with the oxidation of glucose were made with the Warburg technique at three temperatures: 22°, 25° and 28° C. Bacteria growing on the solid medium at 20° were removed with a platinum loop

TABLE II

The growth of smooth sinistral (LS) and of smooth dextral (DS) forms of *Bacillus mycoides* on optically isomeric arginines.

Amino acid	Number of cells in LS	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit	Number of cells in DS	Growth on *d*-arginine if growth on *dl*-arginine is taken as a unit
d-arginine.....	40.1	1.26	66.8	2.15
dl-arginine.....	31.9		31.0	
d-arginine.....	43.5	1.87	45.5	1.38
dl-arginine.....	23.3		33.0	

and a suspension of them was made on a salt solution of the following composition: K_2HPO_4—0.05 per cent, $MgSO_4$—0.03 per cent, NaCl—0.03 per cent. Experiments were made with 0.5 cc. of liquid and the oxidation of 0.5 per cent glucose was studied in the atmosphere of oxygen. Into the side tube of the Warburg vessel was poured a 5 per cent solution of KOH for the absorption of carbonic acid. The experiment at every temperature was always begun with a fresh suspension of the standard concentration. Five Warburg manometers were used simultaneously. One of them served as thermobarometer, two of them contained the suspension of sinistral (LS) bacteria, and two others the suspension of dextral (DS) bacteria. Fifteen minutes were allowed for temperature equilibrium, and then the readings of oxygen consumption were made at 20-minute intervals for 1½ hours. On the basis of these data the mean values of oxygen consumption at different temperatures were calculated.

Figure 8 gives the results of two series of experiments. As is characteristic of biological processes generally, the velocity of respiration rises exponentially with the rise of temperature, and practically in equal degree both in the sinistral and in the dextral strains of *Bacillus mycoides.*

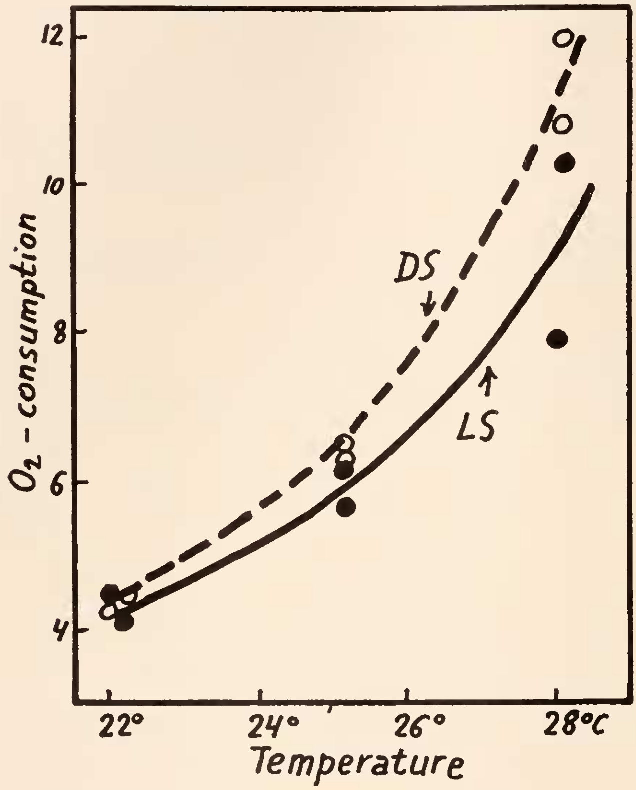

Fig. 8. Oxygen consumption by suspensions of dextral (*DS*) and sinistral (*LS*) strains of *Bacillus mycoides* at different temperatures. Oxygen consumption per 20 minutes is expressed in divisions of the standard manometer and represents an average for four consecutive readings.

Consequently the phenomenon of heat injury in the dextral strain, which is characteristic for its growth, is absent in respiration on glucose.

Discussion

Investigations of different physiological properties of dextral and of sinistral strains of *Bacillus mycoides* show that in the basic features of their metabolism these strains are alike. Both in the sinistral and in the dextral strains natural dextrorotatory arginine proves to be more suitable material for growth than the racemic arginine. Both the sinistral and the dextral strains oxidize natural dextrorotatory glucose equally well and with the same temperature coefficients. The specific difference between dextral strains and sinistral ones is consequently connected not with the inversion of the fundamental protoplasmic system but with differences in some secondary substances determining the cellular form. Castle (1936) has recently undertaken the study of the spiral growth of the cell in *Phycomyces*. He advocates the idea that the spiral structure of the growing cell wall is not strictly predetermined beforehand, but depends on interaction of forces acting in the region of growth. The twist of the growing elastic elements of the wall may be the result of their resistance to the pressure of turgor, as has been shown by Castle (1936) with a special model. It is interesting that if the elastic elements of the wall are distributed symmetrically, in 50 per cent of the experiments with the model dextral spirals are obtained, and in 50 per cent sinistral spirals. As the sinistral direction of spirals is typical for *Phycomyces,* it is evident that the reason for this fact, if one adopts the explanation of Castle, is to be found in some sinistral distribution of elastic elements of the cell wall, which later, under the action of turgor, leads to the formation of sinistral spirals.

As Castle himself points out, the mechanism of spiral growth in different organisms can be different, and one cannot directly transfer his considerations to the bacteria, the more so since the cell wall of the latter consists of some specific protein material which is formed in close connection with the cellular protoplasm (John-Brooks, 1930). It is only important to realize that in the spiral growth of the cell wall there is, first, a system of forces directly inducing the spiral twist (like the turgor in the model of Castle) and, second, that there is some preexisting asymmetric system (distribution of the elastic elements of the wall), the dextrality or sinistrality of which brings on the dextrality or sinistrality of the spiral growth. Whatever the actual mechanism of spiral growth may be, one can conceive the following general scheme of the formation of spiral structure of the cell wall:

(1) Secondary substance of metabolism → (2) Asymmetric structure of the cell wall → (4) The spiral growth

↑

(3) Forces directly inducing the spiral twist (turgor according to Castle)

One may conjecture that the inversion of the direction of spirals in *Bacillus mycoides* is related to optical inversion of some secondary substance in metabolism. The latter brings on the inversion of some structures in the cell wall, and during the interaction of these with the forces inducing the spiral twist the inversion in the direction of the spiral growth of the cells is brought about.

In conclusion it is to be pointed out that the optical inversion of the secondary substances determining cellular form is judged from indirect evidence, and it is consequently highly desirable to check this conclusion by direct chemical methods.

Summary

1. The investigation of different physiological properties of dextral and of sinistral forms of *Bacillus mycoides* shows that in the basic features of their metabolism these strains coincide with one another. Both in sinistral and in dextral strains the natural dextrorotatory arginine proves to be more suitable material for growth than the racemic arginine. Both the sinistral and the dextral strains oxidize the natural dextrorotatory glucose equally well and with the same temperature coefficient.

2. The specific difference between the dextral and the sinistral strains is not consequently connected with the inversion of the basic protoplasmic system, but with differences in some secondary substances determining the cellular form. On the basis of the study of the phenomenon of heat injury in the growth of morphologically inverse dextrally twisted strains of *Bacillus mycoides* the hypothesis is advanced that some organic substance, participating in the determination of the cellular form, has undergone an optical inversion of configuration in the dextral strains.

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